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Developing HPV virus-like particle vaccines to prevent cervical cancer: a progress report

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Abstract

Background: the knowledge that sexually transmitted infection with one of a limited number of human papillomaviruses (HPVs) is a central cause of almost all cervical cancers affords the opportunity to prevent this common cancer through anti-viral vaccination. *Objective:* the spectacular success of vaccines in preventing several other viral diseases offers hope that immunoprophylaxis against the relevant HPVs could lead to a major reduction in cervical cancer incidence. *Results and conclusion:* the results of preclinical studies and early phase clinical trials of virus-like particle (VLP) based subunit vaccines have been very encouraging. However, unique aspects of papillomavirus biology and genital tract infections, and the lack of sexual a transmission model for papillomavirus, make it far from certain that effective prophylactic vaccination against genital HPV infection will be easily achieved. Future clinical efficacy trials will likely test the hypothesis that parenteral injection of VLPs can induce antibody mediated and type specific protection against genital tract HPV infection and subsequent development of premalignant neoplastic disease.

Keywords: Virus-like particle; Human papillomaviruses; Cancer

Greater than 99% of cervical cancers were found to contain human papillomavirus (HPV) DNA is a recent multinational survey of cervical cancers (Walboomers et al., 1999). In addition, there is very strong epidemiological and experimental evidence supporting the etiologic role of HPV infection in the development of cervical cancer (IARC, 1995). Therefore it is almost universally accepted that effective prevention of sexually transmitted infections by the cancer-associated types would dramatically reduce the incidence of cervical cancers. In addition, prophylactic HPV vaccines could potentially decrease the incidence of some other cancers with lower prevalence and/or weaker association with HPV infection, such as anal, vulvar and tonsillar cancers (IARC, 1995).

The effectiveness of licensed anti-viral prophylactic vaccines correlates most strongly with the generation of virus capsid antibodies that prevent virus infection (Robbins et al., 1995). However, it

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was difficult to generate practical immunogens that effectively elicited papillomavirus neutralizing antibodies. It has not been possible to produce large amount of authentic papillomavirus virions in culture cells. Even if authentic virions could be produced, and attenuated, they would be unattractive for use in a prophylactic vaccine because they would contain the oncogenic viral genome. It is very unlikely that such a vaccine would be acceptable for vaccination of healthy adolescents, the ultimate target for a prophylactic vaccine. Simple subunit vaccines based upon denatured polypeptides of the major capsid protein L1 were of limited effectiveness in early animal studies, and they did not produce high titers of neutralizing antibodies (Pilacinski et al., 1986; Christensen et al., 1991). Studies in cattle found that intramuscular injection of native virions protected the animals from experimental challenge with the homologous virus, but not other bovine papillomavirus genotypes (Jarrett et al., 1990). These results supported the concept that PV neutralizing antibodies predominately recognize type specific and conformation dependent epitopes. This concept has been verified in a number of subsequent studies, some of which are described below.

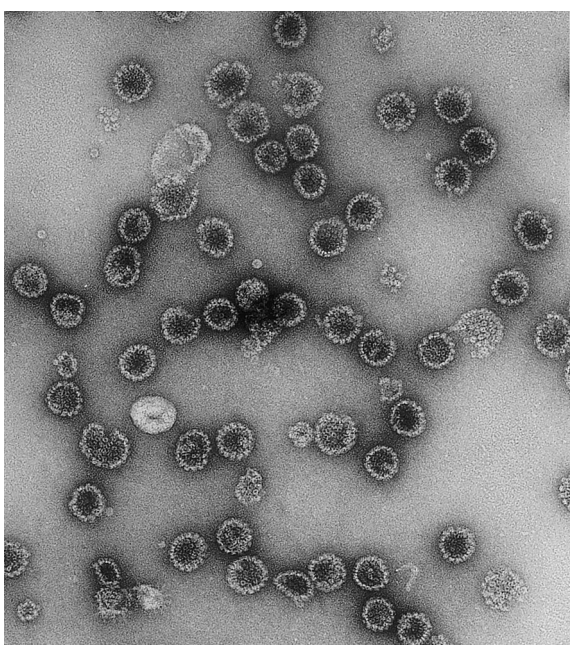

Fig. 1. Transmission electron micrograph of HPV16 L1 VLPs purified from recombinant baculovirus infected Sf-9 insect cells. The VLPs were stained with uranyl acetate and photographed at 36 000 magnification.

The major breakthrough in prophylactic vaccine development was the discovery that L1 can self assemble into virus-like particles (VLPs) when independently expressed at high levels in cultured cells (Fig. 1). These VLPs not only resemble authentic virions morphologically, but they also mimic virions immunologically, in that they induce high titers of neutralizing antibodies to conformational epitopes when injected into animals (Kirnbauer et al., 1992; Rose et al., 1994; Roden et al., 1996a,b; Unckell et al., 1997). Denatured VLPs do not induce neutralizing antibodies (Kirnbauer et al., 1992). The ability to assemble into VLPs is a common feature of all human and animal PV types so far examined, provided that a wild type gene is used. VLPs can assemble when L1 is expressed in mammalian cells, insect cells, yeast, or even bacteria (reviewed in Schiller and Roden, 1996). Since only the L1 gene is introduced in the cells producing the VLPs; the VLPs represent a potentially safe subunit vaccine that do not contain the viral oncogenes and are not infectious (for reviews see Frazer, 1997; Schiller, 1999; Jochmus et al., 1999).

In addition to generating high titers of antibodies that neutralize PV infection in vitro, vaccination has been shown to protect against experimental infection in three animal models. Unfortunately HPVs do not stably infect or cause disease in experimental animals. Consequently animal studies have involved vaccination with animal VLPs and challenge with the corresponding animal PV type. Protection from experimental challenge was demonstrated after parenteral vaccination of cottontail rabbits PV (CRPV) in rabbits (Breitburd et al., 1995; Christensen et al., 1996), canine oral PV (COPV) in dogs (Suzich et al., 1995), and Bovine PV (BPV) type 4 in cattle (Kirnbauer et al., 1996) (Table 1). CRPV is a cutaneous disease model, while COPV and BPV4 are oral mucosal challenge models. In all studies, microgram amounts of VLPs were injected two to three times and challenge with high dose virus was

Table 1
ELISA titers after VLP vaccination in animal studies demonstrating good protection from experimental challenge[a]

Study[b]	Model	Dose-adjuvant (μg)	Schedule (week)	End-point titer	Time[c]
Suzich	COPV-dog	2×20-none	0, 2	<1000	+2 week
Kirnbauer	BPV4-cow	2×150-alum	0, 4	10 000	+2 week
Breitburd	CRPV-rabbit	3×50-alum	0, 2, 4	5000	+1 week
Christensen	CRPV-rabbit	3×50-none	0, 4, 8	10 000, 100	+2 week, +12 month

[a] The data in the table is adapted from the studies cited below.
[b] References, Suzich et al., 1995; Kirnbauer et al., 1996; Breitburd et al., 1995; Christensen et al., 1996.
[c] Time since last vaccination that animals were tested for VLP antibody titers and challenged with homologous virus.

to lightly abraided squamous epithelium, to expose the underlying basal keratinocytes to virus infection. The collective conclusions of the animal trials of VLP vaccination can be summarized as follows. Greater than 90% protection was seen in all three models when challenge occurred within 1 month of the last booster vaccination. Despite a drop in antibody titer (Table 1), substantial protection was also seen when challenge was delayed until 1 year after the last booster vaccination. Protection was type specific. For instance the BPV4 VLP vaccine did not protect rabbits against challenge with CRPV virions. Incorporation of the L2 minor capsid protein into the VLPs did not increase protection, although an extensive protective dose titration was not performed. Protection was seen after VLP vaccination without adjuvant and when the VLPs were complexed with alum. However, no protection was seen when denatured VLPs were injected. Protection could be transferred to naïve animals in immune serum or purified immune IgG, indicating that antibodies are sufficient to confer protection from experimental challenge.

The encouraging results of the animal studies have induced both commercial and public institutions to undertake clinical trials of HPV VLP-based vaccines. The public sector trials, which are sponsored by the National Cancer Institute in collaboration with the National Institute of Allergies and Infectious Disease and the Johns Hopkins University, will be discussed below. A phase I trial of HPV16 L1 VLPs was recently conducted at Johns Hopkins. It was a blinded and placebo controlled dose escalation trial that examined the effects of adjuvant on reactinogenicity and immunogenicity to VLP vaccination. It involved 72 healthy young women and men. An enrollment criterion of four or fewer lifetime sex partners was included to reduce the likelihood that the vaccinees would have HPV16 virion antibodies prior to vaccination. Groups of 12 were randomized into ten VLP and two placebo-vaccinated subjects. Groups received either 10 μg or 50 μg of VLPs, either without adjuvant, with alum, or with MF59, a microemusion adjuvant kindly provided by Chiron (Singh and O'Hagan, 1999), for a total of six groups. The clinical grade VLPs were purified from HPV16 L1 recombinant baculoviruses infected Sf-9 insect cells by Novavax (Rockville, MD). The vaccine was given by intramuscular injection of the deltoid at 0, 1, and 4 months.

Preliminary analysis of the results indicated that all formulations of the vaccine were well tolerated. The predominant reaction noted was local pain at the site of injection that resolved spontaneously within a few days. Greater and more frequent local pain was reported in the vaccinees receiving the formulations containing MF59 than in the vaccinees receiving the other formulations. There were no substantial systemic side effects to the vaccine.

Preliminary analysis of the immune response to vaccination indicated that all vaccinees receiving VLPs seroconverted by 1 month after the second vaccination, as measured in a VLP-based IgG ELISA. None of the placebo vaccinated subject seroconverted during the course of the study. For the 10 μg per dose groups, final geometric mean ELISA titers of sera taken 1 month after the third injection were higher for the groups vaccinated

with VLPs formulated with either adjuvant than for the group vaccinated with 10 μg VLPs in the absence of adjuvant. The higher dose substantially increased the response to the VLPs formulated either without adjuvant or with MF59, but did not increase the response to the VLPs in alum. A similar lack of dose dependency after vaccination with VLPs in alum was previously reported in monkeys using HPV11 VLPs (Lowe et al., 1997). The net result was that the highest geometric mean titers, approximately 10 000, were obtained in the groups vaccinated with 50 μg either alone or with MF59. The magnitude of these titers can be evaluated in the light of two observations. First, they are approximately 50-fold higher than the response seen after natural infection, for instance in the preimmune sera of the study subjects who were seropostive at entry. Second, it is interesting to note that the VLP ELISA titers obtained with the 50 μg dose in the clinical trial compare favorably with the VLP ELISA titers obtained in the animal vaccination studies that demonstrated good protection from high dose experimental challenge, although some caution must be taken in comparing studies with different ELISA protocols. For instance, three injections of 50 μg of CRPV VLPs without adjuvant in rabbits also produced serum ELISA titers of 10 000 (Christensen et al., 1996). Since the formulation that included MF59 induced greater local reactogenicity, it was decided to use 50 μg without adjuvant in an ongoing phase II trial.

Because the ELISA assay measures both virion neutralizing and non-neutralizing antibodies that bind to the VLP preparation, it was important to also specifically evaluate the induction of HPV16 neutralizing antibodies after VLP vaccination. To do this, we used an HPV16 pseudovirion neutralization assay previously developed in the laboratory (Roden et al., 1996a,b). The assay utilities in vitro generated pseudovirions, consisting of an HPV16 L1/L2 capsid surrounding the BPV1 genome. This allows for the evaluation of HPV16 neutralizing antibodies by measuring the ability of immune sera to inhibit pseudovirion mediated focal transformation of mouse fibroblasts in

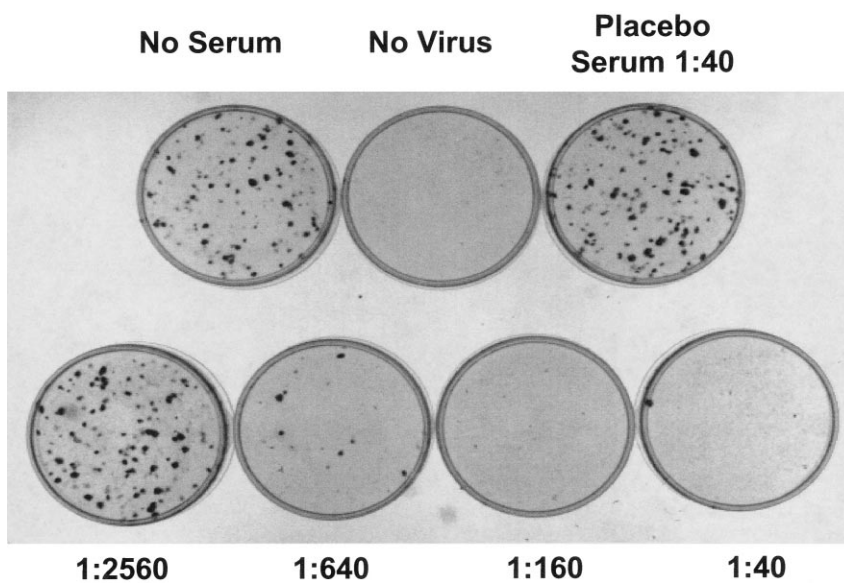

Fig. 2. Neutralization of HPV16 pseudotype virus by human serum after vaccination with HPV16 L1 VLPs. The 'no serum' plate indicates the number of foci obtained with a standard inoculum of pseudovirus in the absence of serum. The 'no virus' plate demonstrates the lack of focal transformation in the absence of the pseudovirus. The 'placebo serum 1:40' demonstrates the absence of neutralizing activity in the serum of a placebo-vaccinated subject at the lowest dilution tested. The bottom row depicts the neutralizing activity of a four-fold serum dilution series from a subject vaccinated three times with 50 μg of HPV16 VLPs without adjuvant. The neutralizing titer of this serum is 640.

monolayer culture (Fig. 2). Preliminary analysis indicated that there was a good correlation between the results in the neutralizing and ELISA assay, both for geometric mean titers of groups and for individual sera within groups. As expected, the neutralizing assay was less sensitive, generating titers that were approximately 20-fold lower than the ELISA titers.

These preliminary analyses of the HPV16 VLP phase I results are encouraging in that they establish that parenteral vaccination with a relatively low dose of VLPs without adjuvant can generate high titers neutralizing serum antibodies in healthy individuals with no indication of substantial adverse side effects. Similar conclusions for diseased individuals were also reached in a recent study involving HPV6b vaccination of genital warts patients (Zhang et al., 2000).

While the early phase clinical trials have been very encouraging, they leave several important question unanswered. The first is whether serum IgG antibodies alone are sufficient for protection against infection at the cervix. Secretory (s)IgA is normally considered to be the first line of antibody defense at mucosal surfaces (van Ginkel et al., 1997). Local genital tract responses were not measured in our phase I trial, in part because the trial was open to both sexes, and because no genital tract sIgA would be expected after systemic VLP vaccination. None was detected after parenteral VLP injection of mice and monkeys (van Ginkel et al., 1997). However, serum IgG could protect against cervical infection by two mechanisms. First, substantial induction of antigen-specific IgG in a woman's genital tract can be induced by parenteral inoculation of a subunit vaccine (Bouvet et al., 1994). This presumably occurs primarily via transudation of serum IgG onto the local mucosal surfaces (van Ginkel et al., 1997). The prospects for effective protection via transudated IgG at the cervix are complicated by the possibility that the levels could vary substantially during the menstrual cycle. VLP specific IgG and sIgA levels were shown to vary inversely during the estrus cycle of mice (Nardelli-Haefliger et al., 1999). A study that involves careful monitoring of genital tract antibodies in VLP vaccinated women throughout a complete menstrual cycle is needed to critically evaluate the likelihood that transudated of IgG will be sufficient to protect women from cervical infection. Second, PV infection of stratified squamous epithilia is thought to require trauma to expose the basal cells to the infecting virus. If infection of intact cervical epitheilium rarely occurs, then exudated serum IgG at a traumatic stite of infection could play a major roll in protection. This is presumably the mechanism through which systemic VLP vaccination protected animals from experimental challenge in the animal vaccine trials.

Although serum IgG may prove protective, it seems worthwhile to investigate possible vaccine strategies that would generate both serum IgG and sIgA. Several methods to induce mucosal immune response after VLP vaccination, including live bacteria and viruses, have been evaluated in animal models (Hagensee et al., 1995; Nardelli-Haefliger et al., 1997). Intranasal and intragastric application of purified VLPs have also been shown to be effective at generating both serum IgG and genital tract IgA in mice. However, a larger dose of VLPs was required to generate a systemic response similar to that seen after parenteral injection (Bamelli et al., 1998; Liu et al., 1998; Rose et al., 1999). It remains to be determined whether these approaches will efficiently and consistently induce both the systemic and local genital tract antibodies in humans. To address this question, clinical trials, involving mucosal application of purified VLPs, are either in progress or in the planning stage.

A second question that remains to be evaluated is the duration of the virion antibody response. In rabbits, serum IgG titers to VLPs declined 100-fold in 1 year, yet significant protection from experimental challenge was seen (Christensen et al., 1996). Since there is no animal model for sexual transmission of a PV to the cervix, it is impossible to critically evaluate the levels of local antibodies required for long lasting protection from natural venereal transmission. This question will need to be addressed in long-term follow up of the anticipated efficacy trials.

It is unfortunate that in vitro analyses of antibodies raised against HPV VLPs strongly suggest that protection from VLP-based vaccines will be

largely type specific (Roden et al., 1996a,b; Unckell et al., 1997; White et al., 1998). Therefore a vaccine that could potentially prevent more than 50% of cervical cancer would have to be multivalent. Including VLPs from types 16, 18, 31, and 45 could potentially prevent up to 80% of cervical cancers worldwide (Bosch et al., 1993). It is hoped that eventually a widely distributed vaccine would eventually contain at least most of these types. The possibility has been raised that protection against one or a few types might simply result in an increase in infections by other types that are also oncogenic and there would be no appreciable decrease in cancer rates, even if the major oncogenic types were eliminated through vaccination. However, a recent analysis of women with multiple infections indicates that infections with different genital HPV types are independent events that do not appreciably impact one another (Liaw et al., 2000). Therefore, unlike the case for bacteria, where there can be competition for colonization of specific anatomical sites, there is no reason to suspect that protection against one type should result in the change in the prevalence of another.

There are excellent reasons also to consider including the VLPs of at least one non-oncogenic type, HPV6, in a prophylactic vaccine. HPV6 appears to cause the majority of genital warts in the US (Greer et al., 1995) and HPV6 VLPs induces antibodies with some cross-neutralizing activity against HPV11 (Christensen et al., 1994), the second most common cause of genital warts. Inclusion of this VLP type in a polyvalent vaccine would address the public health issue of the high incidence of genital warts and the difficulties in treating them. Inclusion of HPV6 VLPs might make the vaccine more attractive for women, especially since it would prevent a more immediate disease than cervical cancer. In addition, it might make a significant difference in the acceptance of the vaccine by men. Men suffer much less frequently from HPV associated cancers than do women, but they have a similar incidence of genital warts as women.

Initial planning for an HPV16 VLP vaccine efficacy trial in the Costa Rica province of Guanacaste has begun. The trial would be sponsored by the National Cancer Institute and the National Government of Costa Rica. This site was selected because it is the site of a large ongoing natural history study of genital HPV infection and its relationship to cervical neoplasia (Schiffman et al., 2000). Thus the target disease is well understood in this setting and much of the infrastructure for conducting the trial is in place. Also, Guanacaste has a relatively high incidence of cervical cancer (Schiffman et al., 2000). This could make the potential benefit to risk ratio more favorable than in many other settings. The primary question to be addressed in this proof of concept trial would be whether simple parenteral injection of purified HPV16 VLPs could protect against acquisition of persistent HPV16 infection and subsequent development of cervical dysplasia. HPV16 was chosen as the target for the trial because it is the most common type in cancers worldwide, and also the type most frequently detected in cervical lesions in Guanacaste (Herrero et al., 2000). A three-arm trial is being considered. In addition to a placebo control arm, one arm would involve intramuscular vaccination with HPV16 L1 VLPs and the other involved vaccination with a chimeric HPV16 VLP. The chimeric VLP would be composed of L1 plus a recombinant protein consisting of HPV16 L2 fused to non-structural HPV16 proteins (most likely both E7 and E2). The latter arm would evaluate the possibility that a vaccine generating cell mediated immune response to non-structural viral proteins might increase vaccine efficacy by inducing regression of subclinical infections that result from imperfect neutralization of virus by antibodies. In the absence of adjuvant, chimeric VLPs have been shown to induce strong cytotoxic T cell responses in vitro (Peng et al., 1998; Rudolf et al., 1999; Schafer et al., 1999) and potent anit-tumor responses in mouse models (Muller et al., 1997; Greenstone et al., 1998). Using protection from HPV16 associated low grade squamous epithelial leasions (LSIL) as the endpoint to power the study, it is anticipated that approximately 3500 young women per arm will have to be followed for 3–4 years to determine if the vaccine is effective. In actuality, all grades of cervical abnormalities, and also persistent HPV16 DNA, will be evaluated as measures of vaccine efficacy. It is

anticipated that an effective prophylactic vaccine will proportionally reduce all measures of HPV16 infection and disease. However, protection against other HPV types is not expected.

In summary, there has been considerable progress in the development of prophylactic HPV vaccines in the 8 years since the discovery of papillomavirus VLPs. Preclinical studies have produced attractive vaccine candidates and the recent early phase clinical trials have yielded exceptionally promising results. However, the lack of a sexual transmission model in animals makes it impossible to confidently predict the outcome of the anticipated efficacy trials. Until efficacy trials determine that simple systemic vaccination with purified HPV VLPs induces long-lasting protection from cervical infection, it seems prudent to continue preclinical studies of alternative vaccine candidates that might be more effective, less expensive, and also more practical for worldwide use.

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